

Qualitative Estimation of the Presence of Bioactive Compound in Centella Asiatica: An Important Medicinal Plant

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Abstract-Qualitative phytochemical analysis of this plant confirms the presence of various phytochemicals like alkaloids, flavonoids, tannins, terpenoids, saponin, steroids, and proteins, reducing sugars, carbohydrates and cardiac glycosides in its methanolic extracts. This paper deals with the significance of these phytochemicals with respect to the role of these plants in traditional medicinal system. This preliminary study draws attention to the need for further studies of the active principles identified in the reported species for the treatment of many diseases in order to understand their mode of action in controlling diseases.

Keywords-Phytochemical; *Centella Asiatica*; Methanolic Extract

I. INTRODUCTION

India is one of the twelve megadiversity countries of the world with a rich diversity of biotic resources. Out of thirty-four hotspots recognized, India has two major hotspots, namely the Eastern Himalayas and the Western Ghats. India harbours about 47, 000 species of plants 17,000 of which are angiosperms (Kapaia VY, 2010). The world is now looking towards India due to its rich biodiversity of medicinal plants and abundance of traditional medicinal systems (Salahuddin A et al., 1998). In traditional societies, nutrition and health care are strongly interconnected and many plants have been consumed both as food and for medicinal purposes (Justin Packia Jacob S et al., 2011). Medicinal plants are natural sources of compounds that can be used against many diseases today (Deshpande HA & Bhalsing SR, 2011). Plants and plant-based medicaments are the basis of many of the modern pharmaceuticals we use today for our various ailments. The discovery of medicinal plants has usually depended on the experience of the populace based on long and dangerous self-experimentation. (Chhetri HP et al., 2008).

Phytochemicals are the natural bioactive compounds found in plants. These phytochemicals work with nutrients and fibers to form an integrated part of defense system against various diseases and stress conditions (Koche D, 2010). The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, steroid, terpenoid, carbohydrate and phenolic compounds (Pascaline J et al., 2011). The *Centella asiatica* is a creeping plant that has long, prostrate, filiform, slender stems with long internodes rooting at nodes. It has simple and long petioled leaves, 1-5 in number from each node, and is reniform oval or orbicular, deeply cordate, 1-7 cm in diameter. The small, inconspicuous flower form in short clusters and purple to white-green flowers, 3-6 in number are arranged in umbels arising from the axils of the

leaves. The fruit is 8 mm long, ovoid, hard with strongly thickened pericarp (Kartnig T, 1998).

Centella asiatica L. has been used as a medicinal herb for thousands of years in India, China, Sri Lanka, Nepal and Madagascar. *Centella asiatica* is one of the chief herbs for treating skin problems, healing wounds, revitalizing nerves and brain cells, hence it is primarily known as a "Brain food" in India. (Singh S et al., 2010)

Centella asiatica, or locally known as pegaga, is a weekly aromatic smelling herb of the family Umbelliferare. (Fig. 1) In India, it is described under the name of Mandukaparni and used in Ayurveda medicine. It has been used as a support for quick healing of small wounds. In addition, it is used in the treatment of skin diseases such as eczema, leprosy, psoriasis, burns, itching and insect bites. In contrast with other medicinal plants, this plant has been subjected to quite extensive experimental clinical investigations due to its ability to heal, to relieve and to recover people from various pains and sicknesses. Of the entire genus *Centella*, only the *asiatica* species are put into commercial use today. The areal parts of the plant are used for medicinal purposes (Zainol NA et al., 2008).

A. Scientific Classification

CLASS : Dicotyledenae
SUBCLASS : Polypetalae
SERIES : Calyciflorae
ORDER : Umbellales
FAMILY : Umbelliferae (Apiaceae)
GENUS : *Centella*
SPECIES : *asiatica* (B. Sathya et al., 2007)



Fig. 1 *Centella asiatica* Linn

The present study was designed to evaluate the bioactive chemical constituents of *Centella asiatica* commonly used in herbal medicine in India.

II. MATERIAL AND METHOD

A Sample Collection and Processing

The plant material of *Centella asiatica* was collected from the Vanita and Dhanvantari nursery of Bhopal and identified taxonomically by Dr. Manish Mishra, Ecosystem Management & Tech. Forestry, Indian Institute of Forest Management, Bhopal (MP). The leaves of the plants were properly washed with tap water and then rinsed with distilled water. The rinsed leaves were shed dried and crushed to obtain powder.

Extraction

A quantity of 100g of the dried powder of *Centella asiatica* extracted with 95% methanol (Merck) using soxhlet was shown in Fig. 2. The soxhletion with methanol was done for one week to obtain extract. After that, the extract was evaporated in water bath at 50°C to obtain crude for phytochemical analysis.



Fig. 2 Extraction by Soxhlet apparatus

B Reagent preparation for quantitative estimation

1% Picric acid: 1ml of picric acid dissolved in 99 ml distilled water.

1% ammonia: 1ml of ammonia dissolved in 99ml distilled water.

1% ammonium chloride: 1g of ammonium chloride was dissolved in 100ml distilled water.

Dragendroff's reagent: It is used for the detection of alkaloids. Boil 14g of sodium iodide with 5.2g basic bismuth carbonate in 50 ml glacial acetic acid for a few minutes. Allow it to stand overnight and filter off the precipitate of sodium acetate crystals. To 40 ml of the red-brown filtrate add 160 ml of ethyl acetate and 1 ml water. Preserve the stock solution in amber-colored bottle. When needed, add 20ml of acetic acid to 10ml of this stock solution and make up to 100ml with water.

Mayer's reagent: it is used for the detection of alkaloids. Solution (a) dissolved 1.36 g mercuric chloride in 60ml of distilled water. (B) Dissolved 5g of potassium iodide in 20ml distilled water. Mix A and B and adjust the volume to 100 ml distilled water.

Millon's reagent: It is used to detect reagent for proteins. Dissolve 1g of mercury in 9 ml of fuming nitric acid, and keep the mixture well cooled during the reaction. When the action is complete, add volume of distilled water.

Molisch' reagent: Dissolve 10g of α -naphthol in 100 ml of 95% alcohol. It is used for detection of carbohydrates.

Ninhydrin reagent: It is used for the detection of amino acids. Prepare 0.1% solution in n-butanol.

Wagner's reagent: It is a general reagent for the detection of alkaloids. Dissolve 1.27g of iodine and 2g of potassium iodide in 5 ml of water and make the volume up to 100ml with distilled water.

Fehling's solution: It is used for the detection of reducing sugars. Dissolve 34.66g of copper sulphate in distilled water and make volume up to 500ml (Solution A). Dissolve 173g of potassium sodium tartrate and 50g of sodium hydroxide in D/W and make volume up to 500ml (solution B). Mix two solutions in equal volume for prior use.

Ferric Chloride (alcoholic): A 5%w/v solution of ferric chloride in 90% alcohol is used for the detection of phenols.

Lead acetate: A 25% basic lead acetate solution is used for the detection of flavonoid.

Salkowski reagent: Prepare fresh before use by mixing 1 ml of 0.5M FeCl_3 in 50 ml of 35% HClO_4 .

C Test for Qualitative Estimation of Bioactive Compounds of *Centella Asiatica*

1) Test for Alkaloids

Methanolic extract was warmed with 2% H_2SO_4 for two minutes. It is filtered and few drops of reagents were added and indicated the presence of alkaloids.

a. Dragendroff's reagent-A red precipitation indicates the positive.

b. Mayer's reagent-A creamy- white colored precipitation positive.

c. Wagner's reagent-A reddish-brown precipitation positive.

d. Picric Acid (1%)-A yellow precipitation positive.

2) Test for Flavonoids

A small quantity of the extracts is heated with 10 ml of ethyl acetate in boiling water for 3 minutes. The mixture is filtered differently and the filtrates are used for the following test.

A. Ammonium Test

The filtrate was shaken with 1 ml of dilute ammonia solution (1%). The layers were allowed to separate. A yellow coloration was observed at ammonia layer. This indicates the presence of the flavonoid.

B. Aluminum Chloride Test

The filtrates were shaken with 1 ml of 1% aluminum chloride solution and observed for light yellow color. It indicated the presence of flavonoid and diluted NaOH and HCl was added. A yellow solution that turns colorless indicated positive.

1) Test for Terpenoids

a) Salkowski Test

The extract was mixed with 2ml of chloroform and concentrate H_2SO_4 (3ml) is carefully added to form a layer. A reddish brown coloration of the interface is formed to show positive result of the presence of terpenoids.

2) Test for Tannins

A small quantity of the extract is boiled with 5 ml of 45% solution ethanol for 5 minutes. Each of the mixture is cooled and filtered. The different filtrates were used to the following test:

b) Lead Sub Acetate Test

1ml of the different filtrate was added with three drops of lead sub acetate solution. A cream gelatinous precipitation indicates positive test for Tannins.

c) Ferric Chloride Test

1ml each of filtrate is diluted with distilled water and added with two drops of ferric chloride. A transient greenish to black color indicates the presence of Tannins.

3) Test for Steroids

2ml of acetic anhydride was added to extract 2ml of H_2SO_4 . The color changed from violet to blue or green in some samples indicated the presence of steroids.

4) Test for Saponins

a) Frothing Test

A small quantity of different extract was diluted with 4 ml of distilled water. The mixture was shaken vigorously and then observed on standing for stable brake.

5) Test for Glycosides

5ml of diluted sulphuric acid was added in extracts in a test tube and boiled for fifteen minutes in a water bath. It was then cooled and neutralized with 20% potassium hydroxides solution. A mixture of 10ml of equal parts of Fehling's solution A and B were added and boiled for five minutes. A more dense red precipitate indicates the presence of glycosides.

6) Test for Reducing Sugar

A small fraction of the extracts were added vigorously with 5ml of distilled water and filtered to the filtrates while equal volumes of Fehling's solution A and B added and were shaken vigorously. A brick red precipitation indicated positive.

7) Test for Carbohydrates

The extract was shaken vigorously with water and then filtered. To the aqueous filtrate was added few drops of Molisch's reagents. Followed by vigorous shaking again, concentrated H_2SO_4 1ml was carefully added to form a layer below the aqueous solution. A brown ring at the interface indicated the positive.

8) Test for Proteins

5ml of distilled water was added into the extracts. This was left to stand for three hours and then was filtered. To 2ml portion of the filtrate was added 0.1ml Millon's reagent. It was shaken and kept for observation. A yellow precipitation indicates the positive.

III. RESULT AND DISCUSSION

The result of the qualitative analysis of *Centella asiatica* is presented in Table 1. The medicinal value of these plants lies in some chemical substances that have a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, terpenoids, carbohydrates and proteins compounds.

TABLE 1: QUALITATIVE ANALYSIS OF THE PHYTOCHEMICAL OF THE *CENTELLA ASIATICA*. + = PRESENCE OF BIOACTIVE COMPOUND - = ABSENCE OF BIOACTIVE COMPOUND

S. no.	Phytochemical	Result
1	Alkaloid	+
2	Flavonoid	-
3	Tannin	-
4	Saponin	+
5	Steroid	+
6	Terpenoid	+
7	Carbohydrate	+
8	Protein	+
9	Glycoside	+
10	Reducing sugar	+

IV. CONCLUSIONS

The plants studied here can be seen as a potential source of useful drugs. It also justifies the folklore medicinal uses and the claims about the therapeutic values of this plant as curative agent and we therefore, suggest further the isolation, identification, purification, characterization and elucidation of the structure of the bioactive compounds of the *Centella asiatica* that would be obtained with a view to obtain useful chemotherapeutic agent.

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